

CLAIMS:

We claim:

- 5 1. A hybrid polypeptide comprising a polypeptide of interest linked to a polymerisable polypeptide by a linker polypeptide, wherein the linker polypeptide comprises a recognition site for a proteolytic agent.
2. A hybrid polypeptide according to claim 1 wherein the proteolytic agent is selected from the following group: 3C-protease from a human rhinovirus type 14 (HRV
10 protease 3C), thrombin, Factor Xa, enterokinase and a chemical capable of proteolytic activity.
3. A hybrid polypeptide according to claim 2 wherein the proteolytic agent is 3C-protease from a human rhinovirus type 14 (HRV protease 3C).
4. A hybrid polypeptide according to claim 1 wherein the recognition site comprises
15 an amino acid sequence selected from the following group:
Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro, Leu-Val-Pro-Arg-Gly-Ser, Ile-Glu-Gly-Arg and Asp-Asp-Asp-Asp-Lys.
5. A hybrid polypeptide according to claim 2 wherein the chemical capable of proteolytic activity is cyanogen bromide.
- 20 6. A hybrid polypeptide according to any one of claims 1 to 5 wherein the linker polypeptide is encoded by a polynucleotide comprising a cloning site.
7. A hybrid polypeptide according to claim 6 wherein the cloning site is a multiple cloning site.
8. A hybrid polypeptide according to any one of claims 1 to 7 wherein the linker
25 polypeptide comprises a spacer polypeptide of sufficient length to allow or enhance

cleavage of the polypeptide of interest from the polymerisable polypeptide, or to avoid unfavourable steric interference between the polypeptide of interest and the polymerisable polypeptide.

9. A hybrid polypeptide according to claim 1 wherein the polypeptides are linked by antibody interaction.
10. A hybrid polypeptide according to claim 9 wherein the antibody interaction is achieved by a process comprising attaching an antibody specific for the polymerisable polypeptide to the polypeptide of interest.
11. A hybrid polypeptide according to claim 9 wherein the antibody interaction is achieved by a process comprising attaching an antibody specific for the polypeptide of interest to the polymerisable polypeptide.
12. A hybrid polypeptide according to claim 9 wherein the antibody interaction is achieved using a bi-specific antibody directed to both the polypeptide of interest and the polymerisable polypeptide.
13. A hybrid polypeptide according to any one of claims 1 to 12 wherein the polymerisable polypeptide is a polypeptide that naturally polymerises with itself.
14. A hybrid polypeptide according to claim 13 the polymerisable polypeptide is tubulin or actin.
15. A hybrid polypeptide according to claim 13 wherein the polymerisable polypeptide is an FtsZ protein or a variant thereof.
16. A hybrid polypeptide according to claim 15 wherein the polymerisable peptide is *E. coli* FtsZ protein or a variant thereof.
17. A hybrid polypeptide according to claim 16 wherein the variant *E. coli* FtsZ protein comprises replacement of the aspartate residue at position 212 of the protein with a cysteine or asparagine residue.

18. A hybrid polypeptide according to claim 16 wherein the variant FtsZ comprises a mutation selected from one of the following: replacement of alanine by threonine at position 70, replacement of aspartate by alanine at position 209 and replacement of aspartate by alanine at position 269.
- 5 19. A hybrid polypeptide according to any one of claims 1 to 18 wherein the polymerisable polypeptide requires an intermediary polypeptide or other molecule in order to polymerise.
20. A hybrid polypeptide according to any one of claims 1 to 19 wherein the polypeptide of interest is of prokaryotic origin.
- 10 21. A hybrid polypeptide according to any one of claims 1 to 19 wherein the polypeptide of interest is of eukaryotic origin.
22. A hybrid polypeptide according to any one of claims 1 to 21 wherein the polypeptide of interest is selected from the group comprising: an endonuclease, a methylase, an oxidoreductase, a transferase, a hydrolase, a lysase, an isomerase, a ligase, 15 a storage polypeptide, a ferritin, an ovalbumin, a transport protein, haemoglobin, serum albumin or ceruloplasmin, an antigen, an antigenic determinant for use in the preparation of vaccines or diagnostic agents, a protective protein, a defence protein, thrombin, fibrinogen, binding proteins, antibodies, immunoglobulins, a human growth hormone, somatostatin, prolactin, estrone, progesterone, melanocyte, thyrotropin, calcitonin, 20 gonadotropin, insulin, a hormone identified as being involved in the immune system, interleukin 1, interleukin 2, colony simulating factor, macrophage-activating factor, interferon, a structural element, collagen, elastin, alpha-keratin, glyco-protein, virus-protein and muca-protein.
23. A hybrid polypeptide according to claim 22 wherein the polypeptide of interest is a 25 protease.

24. A hybrid polypeptide according to claim 23 wherein the protease is 3C-protease from human rhinovirus type 14 (HRV protease 3C).
25. A hybrid polypeptide according to any one of claims 1 to 23 wherein the polypeptide of interest is a synthetic polypeptide.
- 5 26. A method of sequestering and/or purifying a polypeptide of interest comprising the step of polymerising a hybrid polypeptide which hybrid polypeptide comprises the polypeptide of interest linked to a polymerisable polypeptide.
27. A method according to claim 26 wherein the polypeptide of interest is linked to the polymerisable polypeptide by fusing the polypeptide of interest directly to the
- 10 polymerisable polypeptide.
28. A method according to claim 26 wherein the polypeptide of interest is linked to the polymerisable polypeptide by a linker polypeptide.
29. A method according to any one of claims 26 to 28 wherein the hybrid polypeptide is produced *in vivo*.
- 15 30. A method according to claim 26 wherein the hybrid polypeptide is a polypeptide according to any one of claims 1 to 25.
31. A method according to any one of claims 26 to 30 wherein polymerisation is performed under controlled chemical and/or physical conditions.
32. A method according to any one of claims 26 to 31 wherein the polymerisable
- 20 polypeptide is polymerised by the addition of an agent which induces polymerisation.
33. A method according to claim 32 wherein the polymerisation inducing agent is GTP, ATP and/or a cation.
34. A method according to claim 33 wherein the cation is selected from the following group: magnesium, calcium, nickel, cobalt, zinc and manganese.

35. A method according to claim 31 wherein the polymerisable polypeptide is polymerised by a change in temperature.
36. A method according to any one of claims 26 to 35 wherein the polymerised hybrid polypeptide is purified by a first purification step and wherein the first purification step
5 may be the only purification step or may be followed by further purification steps.
37. A method according to claim 36 wherein the first purification step purifies the polymerised hybrid polypeptide by physical techniques discriminating on the basis of size and/or weight.
38. A method according to claim 37 wherein the polymerised hybrid polypeptide is
10 purified by centrifugation, differential sedimentation, filtration, dialysis and/or flow sorting such that the polymerised hybrid polypeptide is isolated.
39. A method according to claim 38 wherein after the first purification step the polymerised hybrid polypeptide is dissociated.
40. A method according to claim 39 wherein dissociation is achieved by removal of
15 the agent which induces polymerisation and/or incubation of the polymerised hybrid polypeptide at a suitable temperature.
41. A method according to claim 39 or claim 40 wherein the dissociated hybrid polypeptide is purified by a second purification step.
42. A method according to claim 41 wherein the second purification step comprises
20 purification of the hybrid polypeptide on the basis of size and/or weight.
43. A method according to claim 41 wherein polymerisation, dissociation and purification of the polymerisable hybrid polypeptide are repeated such that substances larger and smaller than the hybrid polypeptide are removed.
44. A method according to any one of claims 26 to 43 wherein the polymerisable
25 polypeptide is cleaved from the polypeptide of interest by a proteolytic agent.

45. A method according to claim 44 wherein cleavage by the proteolytic agent does not substantially interfere with the biological or chemical activity of the polypeptide of interest or the polymerisable polypeptide.
46. A method according to claim 44 or claim 45 wherein the proteolytic agent is a
5 protease.
47. A method according to claim 46 wherein the protease is linked to a polymerisable polypeptide to form a “protease hybrid polypeptide”.
48. A method according to claim 47 wherein the polymerisable polypeptide to which the protease is linked is identical to the polymerisable polypeptide to which the
10 polypeptide of interest is linked, or is a variant thereof.
49. A method according to claim 47 or claim 48 wherein after cleavage of the polypeptide of interest from the polymerisable polypeptide, the protease hybrid polypeptide is polymerised.
50. A method according to claim 49 wherein the polypeptide of interest is purified
15 from the polymerised protease hybrid polypeptide.
51. A method according to any one of claims 44 to 46 wherein the proteolytic agent is fused to the hybrid polypeptide.
52. A method according to any one of claims 44 to 51 wherein the polymerisable polypeptide released after cleavage from the polypeptide of interest is polymerised.
- 20 53. A method according to claim 52 wherein the polymerised polymerisable polypeptide is removed from the polypeptide of interest by a method which discriminates on the basis of size and/or weight.
54. A method according to any one of claims 46 to 53 wherein the protease is 3C-protease from a human rhinovirus type 14 (HV protease 3C).

55. A method according to any one of claims 26 to 54 wherein the hybrid polypeptide is linked to a support.
56. A method according to claim 55 wherein the support comprises a polymerisable polypeptide.
- 5 57. A method according to claim 56 wherein the support polymerisable polypeptide comprises a polymerisable polypeptide identical to the hybrid polypeptide, or a variant thereof.
58. A hybrid nucleic acid comprising a nucleic acid encoding a hybrid polypeptide according to any one of claims 1 to 25.
- 10 59. A library comprising a plurality of hybrid nucleic acids according to claim 58.
60. A vector comprising a hybrid nucleic acid according to claim 58.
61. A library of vectors comprising vectors according to claim 60.
62. A cell transformed or transfected with a hybrid nucleic acid according to claim 58, a library according to claim 59, a vector according to claim 60, or a library of vectors
- 15 according to claim 61.
63. Cells transformed or transfected with a library according to claim 59 or 61.
64. A library comprising a plurality of hybrid polypeptides according to any one of claims 1 to 25.
65. Use of a hybrid nucleic acid according to claim 58, a library according to claim 59,
- 20 a vector according to claim 60, or a library of vectors according to claim 61 in a method of sequestering and/or purifying a polypeptide of interest.
66. A polypeptide of interest when purified by a method according to any one of claims 26 to 57.
67. A library of polypeptides of interest according to claim 66.
- 25 68. A method of purifying a polypeptide of interest comprising: